Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity[1]

RINA BAROUCH AND MICHAL SCHWARTZ[2]

Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel

SPECIFIC AIMS

Central nervous system (CNS) trauma results in death of severed neurons and eventually of neighboring cells that escaped the initial injury. Systemic injection of T cells directed against CNS myelin-associated proteins such as myelin basic protein (MBP) was shown by our group to result in an increase in the number of T cells that accumulate at the site of injury in an injured nerve. These 'autoimmune' T cells exert a neuroprotective effect by reducing the spread of damage. Thus, modifying the environment of CNS axons by activation of immune cells may stimulate an intrinsic capacity of the neurons to cope with injurious conditions. The mechanism(s) underlying the neuroprotective effect of autoimmune anti-MBP T cells (T_{MBP}) in the injured CNS are not yet fully understood. The aim of this study was to gain a better understanding of the T_{MBP}-induced mechanisms that participate in self repair.

PRINCIPAL FINDINGS

1. Accumulation of B cells in injured optic nerves is significantly increased after injection of anti-MBP T cells

Inbred female Lewis rats received T_{MBP} immediately after unilateral optic nerve crush injury. Control injured rats were injected with PBS. At 4 and 7 days after injury, injured and uninjured (contralateral) optic nerves were analyzed immunohistochemically for the presence of B cells. The number of B cells at and distal to the injury site in the T_{MBP}-treated rats was significantly larger than in the injured or uninjured controls (**Fig. 1**). B cell accumulation in the PBS-treated injured controls was only slightly larger than in the uninjured controls. The accumulation of B cells was transient and decreased after 7 days (Fig. 1).

2. Accumulation of macrophages/microglia in injured optic nerves is significantly increased after injection of T_{MBP}

In both T_{MBP}-treated and PBS-treated optic nerves, an increase in the number of macrophage/microglia was detectable at and distal to the injury site by day 3. By day 4, the increase in the T_{MBP}-treated rats was significantly greater than that in the PBS-treated rats (one-way ANOVA, $P<0.05$).

3. Expression of neurotrophins is transiently increased in injured optic nerves after injection of T_{MBP}

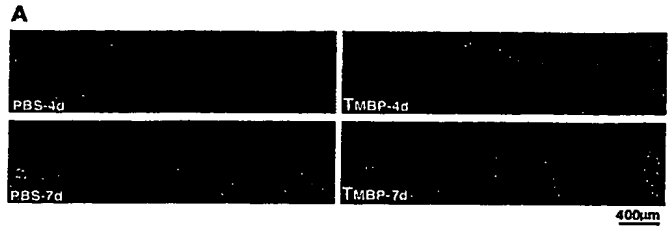
Kinetic studies were performed to determine whether T_{MBP} injection affects the expression of neurotrophins (NTs) at the injury site. As preliminary studies with nerve growth factor (NGF) showed that expression of this NT is maximal 4 days after injury and treatment and decreases after 7 days, all kinetic studies were performed at these times. Maximal expression levels of NGF, brain-derived neurotrophic factor (BDNF), and NT-3 on day 4 were significantly higher in the injured optic nerves of T_{MBP}-treated rats than of PBS-treated rats (**Fig 2**). Seven days later, NT expression was decreased in all of the treated nerves. Expression of NGF in the injured optic nerves was much higher than in the uninjured contralateral nerves, but was lower in the uninjured optic nerves of PBS-treated rats than in those of T_{MBP}-treated rats.

---

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01-0467fje; to cite this article, use *FASEB J.* (June 21, 2002) 10.1096/fj.01-0467fje

[2] Correspondence: Department of Neurobiology, The Weizmann Institute of Science, 76100 Rehovot, Israel. E-mail: michal.schwartz@weizmann.ac.il

0892-6638/02/0016-1304 © FASEB



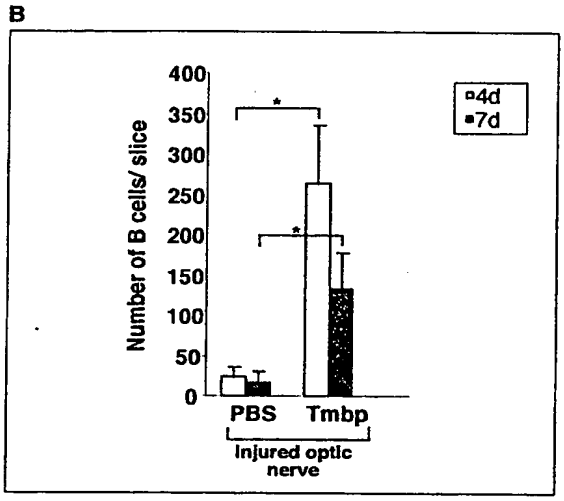


Figure 1. B cell infiltration 4 and 7 days after optic nerve injury. Adult Lewis rats were injected with activated T_{MBP} or with PBS immediately after unilateral crush injury of the optic nerve. After 4 or 7 days, the injured and the uninjured optic nerves were removed, cryosectioned, and analyzed immunohistochemically for the presence of immunolabeled B cells. *A)* Immunolabeled B cells in a representative cryosection taken from the injured nerve of a rat injected with T_{MBP} or PBS 4 or 7 days after injury. *B)* Histograms show the mean numbers of B cells per cryosection (10 mm thick and 6 mm long) ± SE, counted in groups containing three to four rats. Statistical analysis (one-way ANOVA) showed that the number of B cells in the injured optic nerve of rats injected with T_{MBP} was significantly higher than that of rats injected with PBS 4 and 7 days after injury ($P<0.001$). The number of B cells 4 days after injury in the injured optic nerves was significantly higher than that in the uninjured optic nerves of rats injected with T_{MBP} 4 or 7 days after injury ($P<0.001$) or with PBS 4 days after injury ($P<0.05$).

4. Cellular localization of NTs in the injured optic nerve

To determine the cellular distribution of NGF, BDNF, and NT-3, we performed double labeling for T cells, B cells, macrophages/microglia, and astrocytes with anti-NGF, anti-BDNF, and anti-NT-3 antibodies. T cells expressed BDNF and NT-3 but not NGF. B cells expressed all three NTs. Macrophages/microglia expressed NGF and NT-3 but not BDNF. Astrocytes expressed NT-3 but not NGF or BDNF. The same NTs were expressed by the analyzed cells in both the T_{MBP}-treated and the PBS-treated injured optic nerves.

CONCLUSIONS

The results of this study demonstrate that the beneficial autoimmune T cell response to CNS injury is manifested by an increase in the number of B cells and macrophages/microglia and is accompanied by a massive though transient increase in expression of NGF, BDNF, and NT-3. Since NTs are known to play an important role in the post-traumatic maintenance, survival, regeneration, and neuroprotection of neu-

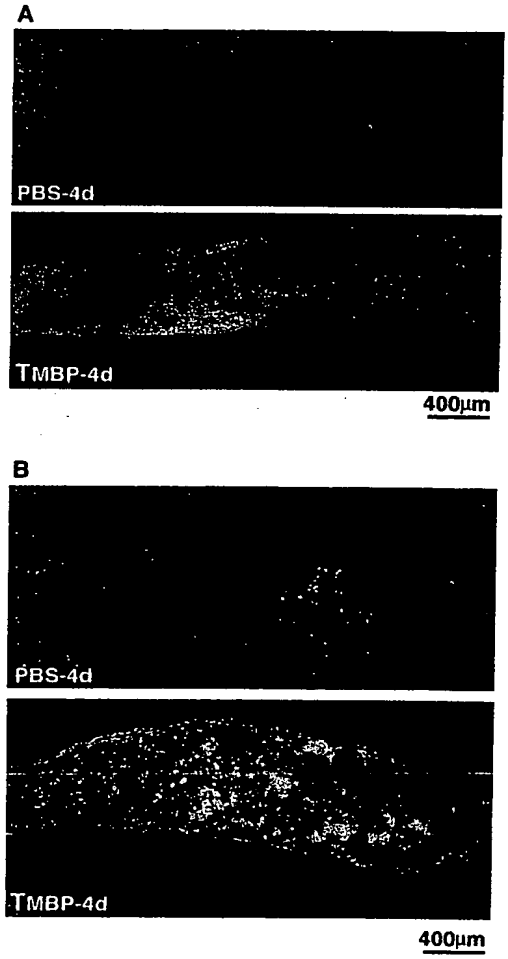


Figure 2. Expression of BDNF and NT-3 by optic nerves. *A)* A representative cryosection stained for BDNF taken from the injured optic nerve of a rat treated with T_{MBP} or PBS 4 days after injury. *B)* A representative cryosection stained for NT-3 taken 4 days after injury from the injured optic nerve of a rat treated with T_{MBP} or PBS.

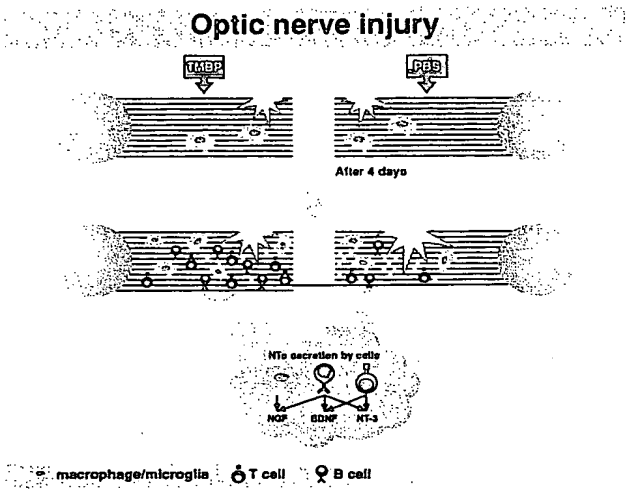


Figure 3. Proposed sequence of events in which the beneficial autoimmune T cell response to CNS injury boosts a local immune response as reflected by an increase in the number of B cells and macrophages/microglia in the injured nerve. The immune response is accompanied by a massive increase in expression of NGF, BDNF, and NT-3 in the injured T_{MBP}-treated nerve. The cellular sources of these NTs are B cells, macrophages, and T cells. Since NTs are known to play an important role in the post-traumatic maintenance, survival, regeneration, and neuroprotection of neurons, it seems likely that the neuroprotective effect of autoimmune T cells is mediated at least in part by NTs.

rons, it seems likely that the beneficial effect of autoimmune T cells is mediated at least in part by NTs. The NTs may influence neuronal survival directly, through binding to their receptors on neurons, or indirectly, by modulating the local immune response, or both.

Our results clearly show that the main cellular sources of the NTs are B cells and macrophages/microglia. NTs are also expressed by T cells and astrocytes but in lower quantities. We further found that the numbers of B cells and microglia/macrophages are significantly increased in injured nerves after passive transfer of T_{MBP}. In view of the present findings, it is reasonable to assume that B cells and microglia/macrophages play a role in mediating some effects of autoimmune T cells and that they do so, at least in part, through secretion of NTs. Other studies have demonstrated, however, that these cells may also have destructive effects on neurons after CNS injury, at least in those animals that are not endowed with the ability to spontaneously evoke protective autoimmunity. We therefore suggest that the beneficial effect of B cells and microglia/macrophages necessitates rigorous control by a suitable T cell regulatory mechanism. FJ